Characterization of extended spectrum β-lactamase (ESBL)-producing *Escherichia coli* in Asi (Orontes) River in Turkey

Cemil Kürekci, Muhsin Aydin, Mustafa Yipel, Mohammad Katouli and Aycan Gündoğdu

ABSTRACT

In this study, the presence of extended spectrum β-lactamase (ESBL)-producing *Escherichia coli* in aquatic environments (the Orontes River and an urban wastewater) was investigated. Fifty-four *E. coli* strains resistant to cefotaxime were isolated from the river waters and nearby waste water treatment plant and screened for ESBL gene variants, different classes of integrons and sulfonamide resistance genes. The ESBL-producing *E. coli* strains were further characterized by PhP-typing system, phylogenetic grouping and antimicrobial susceptibility testing. Of the 54 ESBL-producing strains, 14 (25.9%) belonged to four common PhP types and the remaining were of single types. CTX-M type ESBL genes were identified in 68% of the isolates. The most predominant specific CTX-M subtype identified was $bla_{CTX-M-15}$ ($n = 36$), followed by $bla_{CTX-M-1}$ ($n = 1$). None of the isolates were SHV and OXA positive. Most of the ESBL positive isolates ($n = 37$; 68.5%) were harboring *sul* gene. This study indicates a widespread distribution of CTX-M-15 producing *E. coli* strains in the surface waters in part of Turkey, suggesting an aquatic reservoir for ESBL genes.

Key words | *Escherichia coli*, extended spectrum β-lactamases, Orontes River, PhP-typing

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INTRODUCTION

During the past few decades, antimicrobial resistance has been one of the most important global crises which significantly threaten human health and also add economic burden to health care systems worldwide. Unnecessary and inappropriate uses of antibiotics are considered to be the major contributing factors for the emergence of resistant strains (Berendonk *et al.* 2015). The use of antibiotics in veterinary medicine to prevent infections and to promote growth of livestock adds to the existing problem with the emergence of resistant strains (Smith *et al.* 2002). Apart from clinical settings, antibiotic resistant bacteria have been also shown to be prevalent in aquatic environments including sewage effluents of domestic, municipal and hospitals, groundwater and surface runoffs (Czekalski *et al.* 2012). Animal and human excreta as well as agricultural use are significant sources of pharmaceutical compounds sinking into wastewater systems, which consequently lead to the widespread distribution of antibiotic resistant bacteria in the aquatic environment (Halling-Sørensen *et al.* 1998). In addition, aquatic environments create 'hotspots' for exchange of antibiotic resistance genes among bacterial species with horizontal gene transfer through mobile genetic elements such as integrons, transposons, and plasmids (Moura *et al.* 2010; Amos *et al.* 2014). It has also been suggested that many human pathogens enter into the food chain due to the agricultural utilization of contaminated water sources (Benjamin *et al.* 2013).

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The global dissemination of multidrug resistance among Gram-negative pathogens is becoming increasingly common via clinical specimens, foods of animal origin as well as aquatic environments which pose a dire threat for the community. Among Gram-negative pathogens, extended spectrum beta-lactamase-producing (ESBL) *Escherichia coli* have attracted a lot of attention (Shaikh *et al.* 2015). These strains encode enzymes conferring resistance to a wide variety of β-lactam antibiotics including penicillin, first, second and third-generation cephalosporins, and aztreonam (Malloy & Campos 2011; Gao *et al.* 2015). In the last decade, CTX-M has appeared to be the most dominant ESBL type among clinical isolates where more than 80 different CTX-M enzymes classified into five groups have been described so far (Bonnet 2004). ESBL-producing *E. coli* strains frequently contain resistance genes to other types of antibiotics (e.g. fluoroquinolones, aminoglycosides and tetracycline), which minimizes the availability of effective therapeutic options (Jacoby 2009; Pitout 2010). Over the past few years, ESBL-producing strains of *E. coli* have been frequently isolated from a variety of sources including clinical sources and a wide range of foods as well as occasionally from drinking water sources (De Boeck *et al.* 2012; Altınkum *et al.* 2013; Pehlivanlar-Önen *et al.* 2015). There are numerous studies showing the presence of genes (*bla*TEM, *bla*CTX-M and *bla*SHV) conferring resistance to β-lactam antibiotics in wastewater effluents and rivers around the world (Lu *et al.* 2010; Zurfluh *et al.* 2013).

Also known as Asi, Orontes River is one of the most important rivers in southern Turkey. It originates from Labweh, Lebanon and flows through for about 380 km within three countries and finally pours into the Mediterranean Sea in Hatay province (Göksu *et al.* 2005). The river has been egregiously polluted due to various forms of pollutions, particularly from industrial and agricultural discharges and most importantly untreated effluents from urban wastewater treatment plants (WWTP). Orontes River is the main source for agricultural irrigation as well as fishing for the communities in the area. There are reports of ESBL-producing *E. coli* strains originating from human and animal clinical samples and foods of animal origin in Turkey (Pehlivanlar-Önen *et al.* 2015; Kürekci *et al.* 2016). However, to the best of our knowledge, rivers in Turkey have received little attention in this respect and there no study has been carried out to investigate ESBL-producing strains and the carriage of ESBL types in rivers in Turkey.

The objective of the present study was to characterize the cefotaxime resistant *E. coli* strains isolated from the Orontes River and WWTP in Hatay, Turkey. The presence of ESBL gene variants (bla_{SHV}, bla_{TEM}, bla_{OXA}, and $bla_{CTX\text{-}M}$) and the prevalence of plasmid-encoded sulphonamide resistance genes in these isolates were also examined.

MATERIAL AND METHODS

Sampling

Sewage water samples were collected on three occasions during July, September and December 2014. Samples were collected from the influent and the effluent of a WWTP located in Hatay province with more than 500,000 inhabitants. The WWTP receives sewage (approximately 200–300 L/second) from domestic, hospitals and slaughterhouses in the province and discharges treated sewage (200–300 L/second) directly into Orontes River without disinfection treatment. The WWTP processes influent water through physical treatment including screening, grit removal, gravity sedimentation and trickling filter, followed by secondary sedimentation and discharge treated effluents into Orontes River without chlorination. Water samples of Orontes River were also collected from two locations, i.e. upstream of the Hatay city center (approximately 5 km away from the center) and after passing the city center which is 500 m before the discharge point of the effluent of the WWTP. Water samples were collected in sterile glass bottles, transported to the laboratory in an icebox within 2 h of collection and processed immediately for microbiological analysis.

Isolation and identification of *E. coli*

Membrane fecal coliform (mFC) agar with cefotaxime (2 μg/mL) was used to isolate ESBL-producing *E. coli*. One hundred μL of each sample was inoculated onto mFC agar and incubated at 37 °C for 3 h, then incubated overnight at 44 °C. Up to five suspected *E. coli* colonies (the ones with black color) on mFC agar were chosen and inoculated on blood agar (5% defibrinated horse blood) and incubated at 37 °C for 24 h. The cefotaxime resistant strains were identified as *E. coli* by the Phoenix system and subsequently confirmed by polymerase chain reaction (PCR) amplification of the

universal stress protein (*usp*A) gene as described by Chen & Griffiths (1998). Genomic DNA of these isolates was extracted by using a standard boiling method as described by Anastasi *et al.* (2012). The primer sequences used in the current study and reaction conditions are given in Table 1.

Phenotypic confirmation of ESBL production

A double disc diffusion method was used for the confirmation of ESBL production according to the guidelines described by the Clinical Laboratory Standards Institute (CLSI 2012a). *Klebsiella pneumonia* (ATCC 700603) was used as the standard strain.

Antimicrobial resistance testing

Antimicrobial susceptibility testing of confirmed ESBL positive *E. coli* strains was performed using Phoenix system to determine the minimum inhibitory concentrations (MICs). The following panel of 20 antimicrobials (Phoenix) were used in the current study: amikacin (AM), ampicillin-sulbactam (AMP-SUL), aztreonam (AZT), cefazolin (CFZ), cefepime (FEP), cefoperazone-sulbactam (CEF-SUL), cefoxitin (FOX), ceftazidime (CAZ), ceftriaxone (CTR), ciprofloxacin (CIP), levofloxacin (LEV), colistin (COL), ertapenem (ERT), imipenem (IMI), meropenem (MER), gentamicin (GEN), piperacillin-tazobactam (PIP-TAZ), ticarcillin-clavulanate (TIC-CLA), tigecycline (TIG), and trimethoprim-sulfamethoxazole (STX). In this study, according to MIC values, antimicrobial drug susceptibilities were classified as susceptible, intermediate resistant, and resistant according to the guidelines published by CLSI (2012b). Any intermediate resistant results were counted as susceptible throughout this study.

Typing of isolates

Phylogenetic grouping

In order to exclude homologous isolates, one representative isolate among those with the same resistance pattern and originating from the same source and time points was selected. A total of 54 selected ESBL-producing *E. coli* isolates were examined for their phylogenetic groups (PGGs) using the new quadruplex PCR, as previously reported by Clermont *et al.* (2013).

PhP-typing

All *E. coli* strains ($n = 54$) were typed using PhP fingerprinting method as described by Gündoğdu *et al.* (2011). In this study high-resolution biochemical-fingerprinting PhP-RE plates, which are specifically developed for typing of *E. coli*, were used (Landgren *et al.* 2005). The biochemical fingerprinting values obtained after typing of the isolates were compared pairwise and the similarity between strains was calculated as the similarity coefficient and clustered using UPGMA clustering methods (Sneath & Sokal 1973; Saeedi *et al.* 2005). Isolates having similarity coefficients with each other greater than the default identity level of the software (0.975) were regarded as identical and assigned to the same PhP type. All data handling, including calculations of correlations coefficients, as well as clustering, was performed using the PhPlate software v. 4002 (PhPlate AB). Strains were regarded as belonging to a common clonal group if they had the same PhP type and PGG; otherwise, they were referred to as a single clone.

Identification of ESBL genes

The presence of the bla_{TEM}, bla_{OXA}, bla_{SHV}, and bla_{CTX} genes was tested by PCR as previously described (Ahmed *et al.* 2007). Further, the subtypes of β-lactamase genes were determined by sequencing of the PCR amplicons. The obtained sequencing data were compared to those in GenBank data library using BLASTn program.

Detection of *sul* genes and integron-associated *int* genes

The presence of *sul* genes (*sul*1, *sul*2 and *sul*3) were determined using a multiplex PCR method as described previously by Kerrn *et al.* (2002) and a multiplex PCR was performed to determine the presence of different classes of integron-associated integrase genes (*int*1, *int*2, and *int*3) as described by Dillon *et al.* (2005).

RESULTS

During the initial phase of isolation, a total of 65 cefotaxime resistant *E. coli* strains were recovered from river water

Table 1 | Nucleotide sequence of the primers used in PCR

Targets	Sequence (5′–3′) (amplicon sizes)	PCR conditions	Reference
*chu*A	F: ACGAACCAACGGTCAGGAT R:TGCCGCCAGTACCAAAGACA (Amplicon: 279 bp)		
*yja*A	F: TGAAGTGTCAGGAGACGCTG R:TGGAGAATGCGTTCCTCAAC (Amplicon: 211 bp)	94 °C 5 s 59 °C 15 s (30 cycles)	Clermont *et al.* (2013)
TspE4C	F: GAGTAATGTCGGGGCATTCA R: GCGCCAACAAAGTATTACG (Amplicon: 152 bp)		
*arp*A	F: GATTCCATCTTGTCAAAATATGCC R: GAAAAGAAAAAGAATTCCCAAGAG (Amplicon: 301 bp)	94 °C 5 s 57 °C 20 s (30 cycles)	Clermont *et al.* (2013)
*trp*A	F: AGTTTTATGCCCAGTGCGAG R: TCTGCGCCGGTCACGCCC (Amplicon: 219 bp)	94 °C 5 s 59 °C 20 s (30 cycles)	Clermont *et al.* (2013)
bla_{TEM}	F: ATAAAATTCTTGAAGACGAAA R: GACAGTTACCAATGCTTAATC (Amplicon: 1080 bp)		
bla_{SHV}	F: TTATCTCCCTGTTAGCCACC R: GATTTGCTGATTTCGCTCGG (Amplicon: 727 bp)		
Whole bla_{SHV}	F: CGGCCTTCACTCAAGGATGTA R: GTGCTGCGGGCCGGATAAC (Amplicon: 927 bp)		
bla_{OXA}	F: TCAACTTTCAAGATCGCA R: GTGTGTTTAGAATGGTGA (Amplicon: 610 bp)	95 °C 30 s 56 °C 30 s (35 cycles) 72 °C 30 s	Ahmed *et al.* (2007)
Whole bla_{OXA}	F: GGCAATCCAGCCGGGGCCAA R: CGGGCCTGTTCCCGGGTTAA (Amplicon: 891 bp)		
bla_{CTX-M}	F: CGCTTTGCGATGTGCAG R: ACCGCGATATCGTTGGT (Amplicon: 551 bp)		
Whole bla_{CTX-M}	F: CCAGAATAAGGAATCCCATG R: GCCGTCTAAGGCGATAAAC (Amplicon: 948 bp)		
*sul*1	F: CGGCGTGGGCTACCTGAACG R: GCCGATCGCGTGAAGTTCCG (Amplicon: 433 bp)		
*sul*2	F: GCGCTCAAGGCAGATGGCATT R: GCGTTTGATACCGGCACCCGT (Amplicon: 293 bp)	94 °C 15 s 67 °C 30 s (36 cycles) 72 °C 60 s	Kerrn *et al.* (2002)
*sul*3	F: GAGCAAGATTTTTGGAATCG R: CTAACCTAGGGCTTTGGATAT (Amplicon: 750 bp)		

(*continued*)

Table 1 | continued

Targets	Sequence (5′–3′) (amplicon sizes)	PCR conditions	Reference
int1	F: CAGTGGACATAAGCCTGTTC R: CCCGAGGCATAGACTGTA (Amplicon: 160 bp)		
int2	F: CACGGATATGCGACAAAAAGGT R: GATGACAACGAGTGACGAAATG (Amplicon: 788 bp)	95 °C 30 s 59 °C 30 s (32 cycles) 72 °C 32 s	Gündoğdu *et al.* (2011)
int3	F: GCCTCCGGCAGCGACTTTCAG R: ACGGATCTGCCAAACCTGACT (Amplicon: 979 bp)		
uspA	F: CCGATACGCTGCCAATCAGT R: ACGCAGACCGTAGGCCAGAT (Amplicon: 884 bp)	94 °C 30 s 56 °C 30 s (30 cycles) 72 °C 30 s	Chen & Griffiths (1998)

($n = 30$) and WWTP ($n = 35$) samples and were identified as ESBL producers by phenotypic test according to the CLSI-recommended double-disk diffusion method. Of these, 54 ESBL-producing *E. coli* were included for further studies and the remaining 11 isolates were excluded due to having the same antimicrobial resistance patterns as well as coming from the same samples.

According to the resistance profiling, all ESBL-producing isolates were found to be resistant to AMP-SUL, CFZ, CAZ, and CTR (Figure 1). Fifty-two (96.3%) isolates were resistant to AZT and FEP. The resistance ratios for the remaining antimicrobials were as follows: 37 (68.5%) isolates resistant to CIP, 36 (66.7%) isolates resistant to LEV, 36 (66.7%) isolates resistant to STX, 28 (51.9%) isolates to TIC-CLA, 17 (31.2%) isolates to CEF-SUL, 15 (27.8%) isolates to FOX, 14 (25.9%) isolates resistant to GEN and 13 (24.1%) isolates to PIP-TAZ. None of the isolates were resistant to AM, COL, ERT, IMI, MER and TIG.

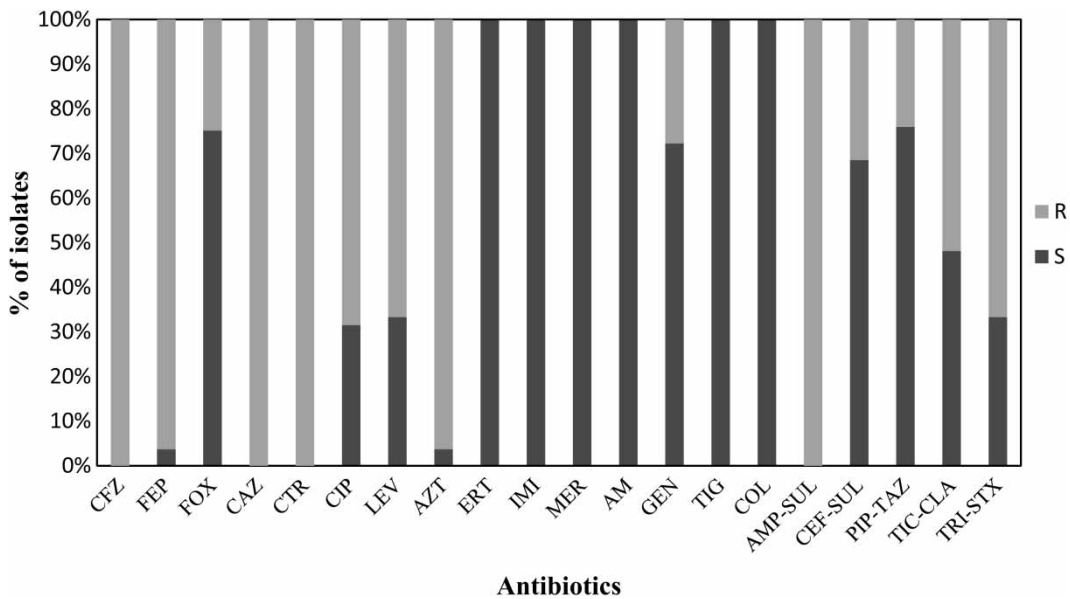

Figure 1 | Antimicrobial resistance percentages among 54 ESBL-producing *E. coli* isolates. Abbreviations; CFZ, cefazolin; FEP, cefepime; FOX, cefoxitin; CAZ, ceftazidime; CTR, ceftriaxone; CIP, ciprofloxacin; LEV, levofloxacin; AZT, aztreonam; ERT, ertapenem; IMI, imipenem; MER, meropenem; AM, amikacin; GEN, gentamicin; TIG, tigecycline; COL, colistin; AMP-SUL, ampicillin-sulbactam; CEF-SUL, cefoperazone-sulbactam; PIP-TAZ, piperacillin-tazobactam; TIC-CLA, ticarcillin-clavulanate; TRI-STX, trimethoprim-sulfamethoxazole.

In river water samples, the most common PGG was group A (52%) followed by group C (16%) and E (16%), and D (8%) and B (8%). Almost a similar pattern was found among *E. coli* strains isolated from WWTP samples. The most common PGG in these samples was group A (48.3%) followed by group C (27.6%), B (10.4%), E (6.9%) and finally D (6.9%) (Table 3). Based on the combination of PhP-typing and phylogenetic grouping, 14 isolates were grouped under four common clonal types (CTs) comprising of between two and five isolates each. The remaining 40 isolates belonged to single clonal types (STs) (Table 2).

According to the PCR and sequencing analysis, among those 54 cefotaxime resistant *E. coli* strains with ESBL phenotype, 20 (37%) possessed $bla_{\text{CTX-M-15}}$ and 16 (29.6%) isolates had $bla_{\text{CTX-M-15}} + bla_{\text{TEM-1}}$, while only one (1.9%) isolate was found to have $bla_{\text{CTX-M-1}} + bla_{\text{TEM-1}}$ (Table 3). In addition, 14 (25.9%) isolates had the $bla_{\text{TEM-1}}$ β-lactamase gene alone. Nevertheless, none of the isolates were found to be SHV or OXA positive.

At least one *sul* gene was found in 37 (68.5%) isolates of which 31 (57.4%) isolates contained both *sul*1 and *sul*2 genes. Additionally, *sul*2 gene alone was found in five (9.3%) isolates and *sul*1 gene alone was found in one isolate. None of the isolates were found to be carrying the *sul*3 gene (Table 3). Thirty-three isolates (61.1%) were found to be positive for the presence of integrase genes. Of these, 31 isolates (57.4%) carried *int*1 and two isolates (3.7%) carried class 2 integron. None of the isolates carried both *int*1 and *int*2 or *int*3 genes (Table 3).

Table 2 | Phenotyping of ESBL-producing *E. coli* by PhP

	Source (number of isolates)			
Types	**OR1 (18)**	**OR2 (7)**	**WW1 (15)**	**WW2 (14)**
CT1 (2)	1	–	1	–
CT2 (5)	1	–	2	2
CT3 (4)	–	1	1	2
CT4 (3)	2	–	–	1
STs	14	6	11	9
Total	18	7	15	14

Locations of the water samples collected: OR1: from upstream of Orontes River; OR2: from Orontes River after passing the city center; WW1: the influent of WWTP; and WW2: the effluent of WWTP.

DISCUSSION

Since their discovery in Western Europe in 1984, ESBL-producing strains have been continually isolated worldwide with increasing frequency among clinical strains as well as isolates from healthy humans and foods of animal origin (Hu *et al.* 2013). In addition, ESBL-producing bacteria have been shown to be widely distributed in the environment, and in particular in soil, plants and water bodies such as rivers, lakes, wastewater effluents and occasionally in drinking water (Hu *et al.* 2013; Zurfluh *et al.* 2013). However, there are not many reports on the persistence of antibiotic resistance of pathogenic bacteria and the molecular basis for resistance phenotype in aquatic environments in Turkey (Toroğlu & Toroğlu 2009; Ozgumus *et al.* 2009). To the best of our knowledge, there is no report related to the ESBL-producing *E. coli* in the rivers and WWTP in this country so far. Hence, the current study for the first time highlights the presence and the importance of ESBL-producing *E. coli* in the Orontes River with the nearby WWTP being the most probable contributing source.

The presence of ESBL-producing *E. coli* strains in surface waters in the current study is in agreement with previous studies focused on the aquatic environments in European countries (Amos *et al.* 2014; Blaak *et al.* 2014), Asian countries (Lu *et al.* 2010; Su *et al.* 2012; Hu *et al.* 2013), Tunisia (Ben Said *et al.* 2016) and Australia (Gündoğdu *et al.* 2013). Interestingly, despite the presence of few CTs of these bacteria in both sources sampled, the majority of ESBL-producing strains belonged to diverse clonal groups based on their PhP-PGG patterns. The PhP system used in this study has been shown to be a highly discriminatory typing method and as powerful as molecular typing methods such as RAPD-PCR (Ramos *et al.* 2010) and ERIC-PCR (Ansaruzzaman *et al.* 2000) for typing of *E. coli* strains. Amos *et al.* (2014) also reported a high genetic diversity among *E. coli* strains carrying $bla_{\text{CTX-M-15}}$ genes in river sediment samples in the UK and suggested that they were of WWTP origin. According to the phylogenetic classification by Clermont *et al.* (2013), the majority of isolates belonged to commensal PGG A and C whereas only a few strains ($n = 4$; 7.4%) belonged to phylogroup D, indicating low prevalence of extraintestinal pathogenic strains in

Table 3 | Characteristics of ESBL-producing *E. coli*

Isolates (n)	Resistance phenotypes*	PGGs	β-lactam gene variant	Types of *int* gene	Types of *sul* gene
Orontes River water samples					
1	FOX, TIC-CLA	E	TEM-1	–	–
1	GEN	A	CTX-M-15	–	–
1	CIP, LEV	A	CTX-M-15	–	–
1	CIP, LEV	C	CTX-M-15	–	*sul*1 + *sul*2
1	CIP, LEV	E	CTX-M-15	–	–
1	TRI-STX	A	TEM-1	*int*1	*sul*1 + *sul*2
1	GEN, TRI-STX	A	CTX-M15, TEM-1	–	*sul*2
1	CIP, LEV, TRI-STX	A	TEM-1	*int*1	*sul*1 + *sul*2
1	CIP, LEV, GEN, TRI-STX	D	CTX-M-15	*int*1	*sul*1 + *sul*2
1	CIP, LEV, GEN, TRI-STX	A	CTX-M-15	*int*1	*sul*1 + *sul*2
1	CIP, LEV, GEN, TRI-STX	E	CTX-M-15	*int*1	*sul*1 + *sul*2
1	CIP, LEV, GEN, TRI-STX	A	CTX-M-15, TEM-1	*int*1	*sul*1 + *sul*2
1	CEF-SUL, FOX, CIP, LEV, TIC-CLA	C	–	–	–
1	CIP, LEV, TIC-CLA, TRI-STX	B	CTX-M-15, TEM-1	*int*1	*sul*2
1	CEF-SUL, PIP-TAZ, TIC-CLA	A	CTX-M-1, TEM-1	–	–
1	CEF-SUL, FOX, CIP, LEV, TRI-STX	B	CTX-M-15	–	*sul*1 + *sul*2
1	CEF-SUL, GEN, TIC-CLA, TRI-STX	A	CTX-M-15, TEM-1	*int*1	*sul*1 + *sul*2
1	CEF-SUL, PIP-TAZ, TIC-CLA, TRI-STX	A	TEM-1	*int*2	*sul*1 + *sul*2
1	CEF-SUL, FOX, CIP, LEV, PIP-TAZ, TIC-CLA	A	CTX-M-15	–	*sul*1 + *sul*2
1	CEF-SUL, FOX, CIP, GEN, TIC-CLA, TRI-STX	D	CTX-M-15, TEM-1	–	*sul*1 + *sul*2
1	CEF-SUL, CIP, GEN, LEV, TIC-CLA, TRI-STX	E	TEM-1	*int*1	*sul*1 + *sul*2
1	CIP, LEV, GEN, PIP-TAZ, TIC-CLA, TRI-STX	A	CTX-M-15, TEM-1	*int*1	*sul*1 + *sul*2
1	CEF-SUL, FOX, CIP, GEN, LEV, TIC-CLA, TRI-STX	C	TEM-1	–	*sul*1 + *sul*2
1	CEF-SUL, FOX, CIP, LEV, MER, PIP-TAZ, TIC-CLA	C	TEM-1	–	–
1	CEF-SUL, FOX, CIP, LEV, PIP-TAZ, TIC-CLA, TRI-STX	A	CTX-M-15	–	*sul*1 + *sul*2
Waste water treatment samples					
1		A	CTX-M-15, TEM-1	–	–
1		B	TEM-1	*int*1	–
1	CIP	A	CTX-M-15	*int*1	–
1	CIP, LEV	D	CTX-M-15	*int*2	–
1	TIC-CLA	D	CTX-M-15, TEM-1	–	–
1	CIP, LEV	C	CTX-M-15	–	–
1	CIP, LEV	C	CTX-M-15	*int*1	–
1	TRI-STX	B	CTX-M-15, TEM-1	*int*1	*sul*1 + *sul*2
1	TRI-STX	A	CTX-M-15, TEM-1	*int*1	*sul*2
1	TIC-CLA, TRI-STX	A	TEM-1	*int*1	*sul*1 + *sul*2
2	CIP, LEV, TRI-STX	A	CTX-M-15	*int*1	*sul*1 + *sul*2
1	CIP, LEV, TRI-STX	A	CTX-M-15	*int*1	*sul*2
1	CIP, LEV, TRI-STX	C	CTX-M-15	–	*sul*1 + *sul*2

(*continued*)

Table 3 | continued

Isolates (n)	Resistance phenotypes*	PGGs	β-lactam gene variant	Types of *int* gene	Types of *sul* gene
1	CIP, LEV, TRI-STX	A	–	*int*1	*sul*1 + *sul*2
1	CIP, GEN, LEV, TRI-STX	A	TEM-1	*int*1	*sul*1 + *sul*2
1	CIP, LEV, TIC-CLA, TRI-STX	A	CTX-M-15, TEM-1	*int*1	*sul*1 + *sul*2
1	CIP, GEN, LEV, TIC-CLA	C	CTX-M-15, TEM-1	*int*1	–
1	CEF-SUL, TIC-CLA, TRI-STX	E	CTX-M-15, TEM-1	*int*1	*sul*2
1	CEF-SUL, TIC-CLA, TRI-STX	B	TEM-1	*int*1	*sul*1
1	CIP, GEN, LEV, TIC-CLA, TRI-STX	A	CTX-M-15, TEM-1	*int*1	*sul*1 + *sul*2
1	CIP, GEN, LEV, TIC-CLA, TRI-STX	A	–	*int*1	*sul*1 + *sul*2
1	CEF-SUL, CIP, LEV, PIP-TAZ, TIC-CLA	C	CTX-M-15, TEM-1	*int*1	–
1	FOX, CIP, LEV, PIP-TAZ, TIC-CLA, TRI-STX	C	CTX-M-15	*int*1	*sul*1 + *sul*2
1	FOX, CIP, LEV, PIP-TAZ, TIC-CLA, TRI-STX	E	TEM-1	–	*sul*1 + *sul*2
1	FOX, CEF-SUL, CIP, GEN, LEV, PIP-TAZ, TIC-CLA	C	TEM-1	–	–
1	CEF-SUL, FOX, CIP, LEV, PIP-TAZ, TIC-CLA, TRI-STX	A	TEM-1	*int*1	*sul*1 + *sul*2
1	CEF-SUL, FOX, CIP, GEN, LEV, PIP-TAZ, TIC-CLA, TRI-STX	C	–	–	*sul*1 + *sul*2
1	CEF-SUL, FOX, CIP, GEN, LEV, PIP-TAZ, TIC-CLA, TRI-STX	A	CTX-M-15	*int*1	*sul*1 + *sul*2

*Resistance phenotypes for the antimicrobials tested apart from the cephalosporin and monobactam; AMP-SUL, ampicillin-sulbactam; AZT, aztreonam; CFZ, cefazolin; FEP, cefepime; CEF-SUL, cefoperazone-sulbactam; FOX, cefoxitin; CAZ, ceftazidime; CTR, ceftriaxone; CIP, ciprofloxacin; GEN, gentamicin; LEV, levofloxacin; PIP-TAZ, piperacillin-tazobactam; TIC-CLA, ticarcillin-clavulanate; TRI-STX, trimethoprim-sulfamethoxazole.

water samples. This is consistent with the results of a recent study on ESBL-producing *E. coli* strains obtained from rivers and lakes in Switzerland (Zurfluh *et al.* 2013). It has to be mentioned however, that the most common phylogroup in our river samples, i.e. PGG A, has been determined as the least abundant group in foods of animal origin in Turkey (Pehlivanlar-Önen *et al.* 2015).

Examining the antibiotic resistance profiles of 54 ESBL-producing *E. coli* strains, it was found that more than 65% of the ESBL-producing *E. coli* isolates were resistant to one of the three antibiotics, i.e. CIP, LEV, and STX. This is not surprising as ESBL-producing *E. coli* have been shown to be commonly resistant to different antibiotic classes, particularly to quinolone and aminogylcoside and sulphonamides due to the carriage of resistance genes on the same conjugative plasmids (Wang *et al.* 2013). A recent study carried out in the Netherlands has reported that ESBL-producing *E. coli* isolated from recreational water were resistant to nalidixic acid (60%), CIP (42%) and cefoxitin (3%) (Blaak *et al.* 2014). As shown in another study (Lu *et al.* 2010), PIP-TAZ and CEF-SUL have been the most potent ESBL enzyme inhibitors with 24.1 and 31.2% resistance, respectively, while AMP-SUL was the least efficient with 100% resistance. All ESBL-producing *E. coli* strains were sensitive to carbapenems, which is of great importance as these molecules are one of the last resort antibiotics for ESBL-producing bacteria for humans. Similar data have been reported for the high susceptibility of *E. coli* to carbapenems (Diallo *et al.* 2013; Blaak *et al.* 2014).

The most common ESBLs genes among our *E. coli* strains was the CTX-M group found in 68% of the isolates. This was not surprising as these enzymes are the most predominant ESBLs among *E. coli* strains isolated from human clinical samples (Altınkum *et al.* 2013) as well as foods of animal origin (Pehlivanlar-Önen *et al.* 2015; Kürekci *et al.* 2016). Widespread dissemination of *E. coli* carrying CTX-M enzyme has also been reported in wastewater, surface water and river sediments worldwide. In a detailed study, Zurfluh *et al.* (2013) investigated the occurrence of ESBL-producing Enterobacteriaceae in rivers and lakes in Switzerland and showed a strong correlation between the strains isolated from clinical samples, healthy human and food producing animal isolates with the environmental isolates in this country. In our study CTX-M-15 was found to

be the most common ESBL type, which is consistent with the findings reported from England and the Netherlands (Amos *et al.* 2014; Blaak *et al.* 2014). For example, Amos *et al.* (2014) found CTX-M-15 type as the predominant ESBLs detected in *E. coli* from a river in England, which was linked to the high abundance of CTX-M-15 type in the effluent of WWTP. Blaak *et al.* (2014) also reported a similar finding on widespread dissemination of CTX-M enzyme, with CTX-M-15 being the most dominant type in recreational water in the Netherlands. The predominance of CTX-M-15 type in the current study could also be explained by selective enrichment with cefotaxime. In fact it has been shown that isolates carrying CTX-M-1 group ESBLs were significantly more resistant to CAZ and cefepime when compared to other CTX-M groups (Hu *et al.* 2013). On the other hand, Korzeniewska & Harnisz (2013) only found OXA-1 and TEM-1 types in their study in Poland, but did not detect CTX-M types.

A combination of trimethoprim and sulphamethoxazole is an important synthetic antimicrobial agent used for the treatment of urinary tract infections caused by *E. coli* (Grape *et al.* 2003). Sulfonamides, alone or in combination with other antimicrobial compounds, have been widely used to treat various infectious diseases caused by bacteria, toxoplasma, and protozoa in animas as well as growth promoting agents in swine production (Prescott 2013). Resistance to sulfonamides among *E. coli* strains is mediated either through the mutations in the chromosomal dihydropteroate synthase gene (*folP*) or through the acquisition of an alternative DHPS gene (*sul*) (Sköld 2000) for which three *sul* genes (*sul*1, *sul*2 and *sul*3) have been described so far (Prescott 2013). We previously found that 90% of the *E. coli* strains carrying *sul* genes in surface waters are similar to those obtained from uropathogenic *E. coli* strains (Gündoğdu *et al.* 2011). In the present study, 68.5% of ESBL positive isolates were found to have *sul* genes (*sul*2; 66.6% and *sul*1; 59.2%). These results are not surprising since DHPS gene mediated sulphonamide resistance has become common among *E. coli* isolates from clinical samples, food producing animals and foods of animal origins (Sköld 2000; Soufi *et al.* 2011). We found that 61% of isolates carried integrase genes, with the majority carrying *int*1. Detection of class I integrons varies in different studies and ranges between 64% (Ben Said *et al.* 2016) and 41% in surface waters (Chen *et al.* 2011). The presence of *E. coli* strains carrying integrin classes 1 and 2 has also been reported in rivers in the northern region of Turkey (Ozgumus *et al.* 2009).

CONCLUSIONS

In conclusion, we found a high level of CTX-M-15 type ESBL enzyme together with *sul* genes among our isolates, indicating the importance of the Orontes River as a potential reservoir of antimicrobial resistance genes. We also found a high diversity of ESBL-producing *E. coli* strains in both the river and WWTP samples which point at the diversity of sources of contamination of the Orontes River in this country.

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AUTHOR DISCLOSURE STATEMENT

The authors declare that there is no conflict of interest with the organization that sponsored this research and publications arising from this research.

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